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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Before Application of: Ester FRIDE et al.

Application No.: 09/698,071 Group Art Unit: 1614

Filed: October 30, 2000 Examiner:

For: AGONISTS SPECIFIC FOR THE PERIPHERAL CANNABINOID RECEPTOR Attorney Docket No.: 7754-071

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Sir:

Applicants have claimed foreign priority benefits under 35 U.S.C. § 119(a)-(d) of Application No. 132661filed on October 31, 1999 in Israel. In support of this claim, a certified copy of said application is submitted herewith.

No fee is believed to be due for this submission. Should any fees be required, however, please charge such fees to Pennie & Edmonds LLP Deposit Account No. 16-1150.

Respectfully submitted,

1/05/01
Date

Allan A. Fanucci, Reg. No. 30,256

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חוק הפטנטים, תשכ"ז - 1967
PATENT LAW, 5727 - 1967

בקשה לפטנט
Application for Patent

לשימוש הלשכה
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מספר: Number	132661
תאריך: Date	31 -10- 1999
הוקדם/נדחה Ante/Post-dated	

אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
(Name and address of applicant, and in case of body corporate-place of incorporation)

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(Hebrew)

AGONISTS SPECIFIC FOR THE PERIPHERAL CANNABINOID RECEPTOR
(באנגלית)
(English)

מבקש בזאת כי ינתן לי עליה פטנט hereby apply for a patent to be granted to me in respect thereof.

*בקשת חלוקה - Application of Division	*בקשת פטנט מוסף - Application for Patent Addition	*דרישה דין קדימה Priority Claim		
מבקשת פטנט from Application	*לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
מס'..... No.....	מס'..... No.....			
מיום..... dated.....	מיום..... dated.....			

* יפוי כח: כללי /-מיוחד - רצוף בזה / עוד יוגש
P.O.A.: general / individual-attached / to be filed later -

הוגש בעניין.....
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תיקנו 2/009

Dr. C. Webb ד"ר ס. וב
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חתימת המבקש
Signature of Applicant

For the Applicants,

Dr. C. Webb
Patent Attorney

היום 31 בחודש אוקטובר שנת 1999
This of the year

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AGONISTS SPECIFIC FOR THE PERIPHERAL CANNABINOID RECEPTOR

אגוניסטים ספציפיים לקולטנים הקנבינואידיים הפריפרליים

AGONISTS SPECIFIC FOR THE PERIPHERAL CANNABINOID RECEPTOR

FIELD OF THE INVENTION

The present invention relates to non-psychotropic cannabinoids that are specific agonists of the peripheral cannabinoid receptor CB2. More particularly, the invention relates to pharmaceutical compositions comprising 4-phenyl pinene derivatives which are specific CB2 agonists useful in therapy of hypertension, inflammation and pain, and gastrointestinal disorders.

BACKGROUND OF THE INVENTION

Two cannabinoid receptors have been identified: CB1, present in the central nervous system (CNS) and to a lesser extent in other tissues, and CB2 present outside the CNS, in peripheral organs including peripheral nerve terminals.

Cannabis sativa preparations have been known as therapeutic agents against various diseases for millenia (1). The native active constituent, Delta 9-tetrahydrocannabinol (delta-9-THC) (2), is prescribed today, under the generic name Dronabinol, against vomiting and for enhancement of appetite, mainly in AIDS patients. However, separation between the therapeutically undesirable psychotropic effects from the clinically desirable ones has not been reported with agonists that bind to cannabinoid receptors. THC, as well as the two major endogenous compounds identified so far that bind to the cannabinoid receptors, anandamide (3) and 2-arachidonylglycerol (2-AG) (4,5) produce most of their effects by binding to both the CB1 and CB2 cannabinoid receptors.

The CB1 receptor is present in the CNS (6,7), and to a lesser extent in other tissues. The CB2 receptor is not present in the CNS, but mostly in peripheral tissue associated with immune functions, including macrophages

and B cells (8), as well as in peripheral nerve terminals (9). (For recent reviews see refs. 10-13). While the effects mediated by CB1, mostly in the CNS, have been thoroughly investigated (11,13), those mediated by CB2 are not well defined.

US Patent 5,434,295 discloses a family of novel 4-phenyl pinene derivatives, and teaches how to use said novel compounds in pharmaceutical compositions useful in treating various pathological conditions associated with damage to the central nervous system. That disclosure neither teaches nor suggests that any of those are selective for peripheral cannabinoid receptors.

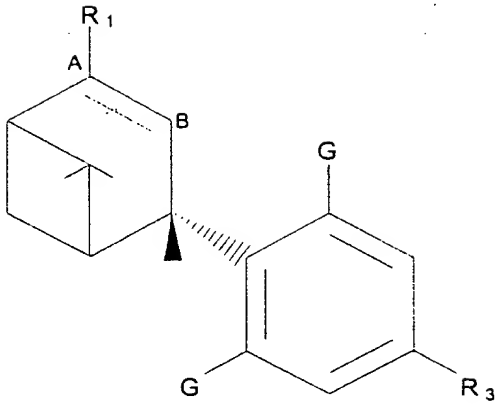
Several synthetic cannabinoids have been shown to bind to the CB2 receptor with a Higher affinity than to the CB1 receptor (as reviewed in 22). Most of these compounds exhibit only modest selectivity (23, 24). However one of the described compounds, a classical THC-type cannabinoid, L-759,656, in which the phenolic group is blocked as a methyl ether, has a CB1/CB2 binding ratio > 1000 (23). The pharmacology of those known agonists has yet to be described.

SUMMARY OF THE INVENTION

It is an object of the present invention to isolate the effects mediated by peripheral cannabinoid receptors CB2, thereby enabling the introduction of new therapeutic entities by preparation of specific CB2 agonists. It is another object of the present invention to provide a CB2 specific agonist, capable of exerting its CB2 specific effects in vivo. According to another aspect of the present invention, methods are provided for use of a CB2 specific agonist in the preparation of a medicament for the treatment of conditions or diseases mediated by this receptor. According to yet another aspect of the present invention methods are provided for the treatment or prevention of diseases by administration to an animal in need thereof of a pharmaceutical composition containing as an active ingredient a therapeutically effective amount of a CB2 specific agonist.

The active ingredient of the pharmaceutical compositions according to the present invention is a compound of the general formula 1:

Formula 1



wherein A---B designates an optional double bond,

R_1 designates a variety of organic moieties,

G designates hydrogen, halogen or various ether groups,

and R_3 designates various alkyl groups, ether groups,

or combinations thereof.

In its broadest aspect the present invention encompasses compounds of Formula 1 as disclosed in US Patent 5,434,295, the teachings of which are incorporated herein in their entirety by reference. The compounds according to said formula that are contemplated herein have the (3S,4S) configuration and are essentially free of the (3R,4R) enantiomer.

Certain compounds of the above formula are novel and in themselves constitute an aspect of the present invention. These compounds include those in which G is hydrogen.

According to currently more preferred embodiments, G hydrogen or is OR_2 where R2 a lower alkyl group of 1 to five carbon atoms.

According to one currently most preferred embodiment, we now disclose the synthesis and utility of such a specific ligand, designated herein as HU-308, its differential binding to CB-1 and CB-2, and its action on several in vivo assays, known to be affected by cannabinoids. HU-308 is a compound of the general formula 1 wherein the substituents are as follows: R_1 is CH_2OH, G is methoxy, and R3 is 1,1 dimethylheptyl.

This new cannabinoid does not bind to CB1 (Ki > 10 mM), but does bind efficiently to CB2 (Ki = 22.7 + 3.9 nM). It shows no activity in a tetrad of behavioral tests in mice which together have been shown to be specific for tetrahydrocannabinol (THC) - type activity in the CNS mediated by CB1. However, HU-308 reduces blood pressure, blocks defecation and elicits antiinflammatory and peripheral analgetic activity.

The hypotensive, antiinflammatory, peripheral analgetic activity and gastrointestinal effects produced by HU-308 are blocked by the CB2 antagonist SR 144528, but not by the CB1 antagonist SR 141716A.

Accordingly, the present invention establishes the principles of the invention by providing novel non-psychotropic cannabinoids that enable new therapies for hypertension, inflammation, pain, and gastrointestinal diseases

BRIEF DESCRIPTION OF THE FIGURES

To assist in the understanding of the invention and, in particular, of the data that is given in the Examples, the following drawings and figures are presented herein:

Scheme 1 shows the synthetic scheme for producing HU-308.

Figure 1 Binding of HU-308 to the CB2 cannabinoid receptor measured by competetive inhibition of [3H]HU-243 in COS-7 cells transfected with plasmids containing the CB2 receptor gene (4).

Figure 2 Absence of psychoactive cannabinoid effect of HU-308. Mice (female C57/BL6) were tested, 2.5 hrs after i.p. injection of HU-308 (40 mg/kg), in the 'tetrad' of tests for cannabinoid activity: Ambulation (a) and rearing (b) in open field; immobility on a ring ('catalepsy', c); hypothermia (d) and analgesia on a hot plate (e). It should be noted that no histopathological damage was observed when mice were kept for up to 60 s on a 59 oC hot plate (35). Open bars, vehicle-treated; closed bars, HU-308 (50 mg/kg).

Figure 3 Intestinal immotility after HU-308 administration. Mice (female Sabra) were injected with HU-308 (20, 50 or 100 mg/kg i.p.). Intestinal immotility was assessed every 15 min, (over a 2 hr period) by the cumulative number of fecal pellets voided in a two hr period after separation into individual cages. After 75 min, mice which had received 100 mg/kg, had voided significantly fewer boli than controls. By 90 min all treatment groups differed significantly from controls. The data presented here reflect the

number of fecal pellets voided over 2 hours after HU-308 administration.

*) significantly less than controls ($p<0.05$)

**) significantly less than controls ($p<0.01$)

Figure 4 Hypotensive effects of HU-308. Anesthetized rats were cannulated. Baseline blood pressure was recorded before HU-308 was injected i.v. (30 mg/kg) (Lower doses did not have significant effects). In experiments with antagonists, SR141716A (3 mg/kg), or SR144528 (1 mg/kg) were injected 5 min prior to HU-308.

*) significantly different from baseline value of controls ($p<0.05$)

Figure 5 Effects of HU-308 and indomethacin on arachidonic acid (A'A)-induced swelling of the ear. Mice (female Sabra) were treated with 4.5 mg A'A (in 5 ml EtOH) dispersed on the inner surface of one of the ears. The other ear was treated with 5 ml of EtOH. Ear swelling was assessed by measuring ear thickness with a dial thickness gauge (Mitutoyo, Japan) just before treatment and every 15 min after A'A application for 90 min.

a) Time curve illustrating that peak swelling of the ear was achieved at about 30 min after A'A application. Vehicle, HU-308 (50 mg/kg) or indomethacin (20 mg/kg) were injected i.p. 60 min before A'A. (Injection of HU-308 30 or 90 min before A'A yielded similar results)

b) Effects of CB1 and CB2 receptor antagonists on the antiinflammatory

effect of HU-308. The CB1 antagonist (SR141716A, 5 mg/kg) or the CB2 receptor antagonist (SR144528, 1 mg/kg) were injected i.p. This was followed 60 min after by HU-308 administration (50 mg/kg i.p.). A'A was administered 50 min after HU-308.

The results are presented as the difference between the A'A treated ear and the EtOH-treated ear. N=5 for each treatment group.

*) significantly different from vehicle-treated mice ($p<0.05$)

**) significantly different from vehicle-treated mice ($p<0.01$)

***) significantly different from vehicle-treated mice ($p<0.001$)

Figure 6 Effects of HU-308 w/o SR144528 on formalin-induced peripheral pain. Vehicle or SR144528 was injected 15 min before an injection of Vehicle or HU-308 (50 mg/kg, i.p.). Ninety min after the injection of HU-308 formalin was injected subcutaneously into the foot. Pain was assessed as the total number of licks of the injected hindpaw recorded for the duration of one hr.

Early (5 min) and late (25-60 min) phase pain were observed as described (20). However, HU-308-induced effects were only observed during the late phase. Hence only data collected at 30 min after formalin injection are presented herein.

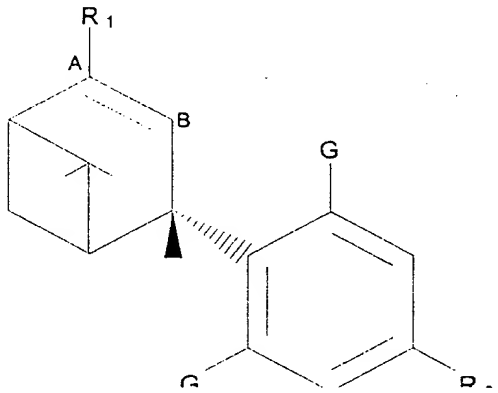
DETAILED DESCRIPTION OF THE INVENTION

Selective agonists for a specific receptor are of interest and importance

as they make possible biochemical and pharmacological investigations of individual receptors and may serve as drugs or drug leads.

The present invention provides novel medicinal uses for the pinene derivatives as shown herein. These compounds have unexpectedly been shown to be CB2 specific agonists.

These methods involve the use of appropriately formulated pharmaceutical compositions with CB2 agonist activity which contain as their active ingredient a compound of the formula:



In the above formula, the dotted line A-----B indicates an optional bond. The variable substituents R_1, G, and R_3 are defined as follows:

R_1 is (a) -R'N(R")$_2$ wherein R' is C_1-C_5 alkyl and each R", which may be the same or different, is hydrogen or C_1-C_5 alkyl optionally containing a terminal -OR'" or -OC(O)R'" moiety wherein R'" is hydrogen or C_1-C_5 alkyl, (b) -Q wherein Q is a heterocyclic moiety having a labile hydrogen atom so that said moiety acts as a carboxylic acid analogue, (c) -R'X wherein R' is C_1-C_5 alkyl and X is halogen, (d) -R'C(O)N(R")$_2$ wherein R' is a direct bond or C_1-C_5 alkyl and each R", which may be

the same or different, is hydrogen or C_1-C_5 alkyl optionally containing a terminal -OR''' or -OC(O)R''' moiety wherein R''' is hydrogen or C_1-C_5 alkyl, (e) -R'C(O)OR'' wherein R' is a direct bond or C_1-C_5 alkyl and R'' is hydrogen or C_1-C_5 alkyl optionally containing a terminal -OR''' or -OC(O)R''' moiety wherein R''' is hydrogen or C_1-C_5 alkyl, (f) -R' wherein R' is C_1-C_5 alkyl, or (g) -R'OR''' wherein R' is C_1-C_5 alkyl and R''' is hydrogen or C_1-C_5 alkyl,

G is hydrogen, halogen, or -OR_2 wherein R_2 is R'', wherein R'' is hydrogen or C_1-C_5 alkyl optionally containing a terminal -OR''' or -OC(O)R''' moiety wherein R''' is hydrogen or C_1-C_5 alkyl, -C(O)OR''' wherein R''' is as previously defined, or -C(O)R''' wherein R''' is as previously defined, and

R_3 is (a) C_1-C_{12} alkyl, (b) -OR'''', in which R'''' is a straight chain or branched C_2-C_9 alkyl which may be substituted at the terminal carbon atom by a phenyl group, or (c) -$(CH_2)_n$OR''' wherein n is an integer of 1 to 7 and R''' is hydrogen or C_1-C_5 alkyl.

The compounds according to said formula that are contemplated herein have the (3S,4S) configuration and are essentially free of the (3R,4R) enantiomer.

Certain compounds of the above formula are novel and in themselves constitute an aspect of the present invention. These compounds include those in which **G** is hydrogen.

We now disclose the synthesis and use of a new type of CB2 specific agonist, having the general formula 1, as defined above. The principles of the invention are exemplified herein by the currently most preferred bicyclic HU-308 (Scheme 1), with a Ki = 22.7 + 3.9 nM (Fig 1). It does not bind to CB1 (Ki > 10 mM). This difference in binding is

reflected in the results of the pharmacological assays. In mice, a high dose of HU-308 (40 mg/kg) did not decrease the activity in an open field trial, did not cause catalepsy, did not reduce body temperature and did not cause analgesia, measured on a hot plate when tested 10, 30 (data not shown) or 150 min after i.p. administration (Fig 2). Such effects are considered to be mediated by the CB1 receptor (11,13,15).

Inhibition of gastrointestinal activity has been observed after administration of Delta 9-THC (25,26,27 and ref therein) or of anandamide (28,29). This effect has been asumed to be CB1-mediated as the specific CB1 antagonist SR 141716A blocked the effect (29). A previous report from our laboratory (28) however suggested that inhibition of intestinal motility may also have a CB2-mediated component. The results exemplified hereinbelow now strongly support this assumption.

HU-308 caused complete inhibition of intestinal mobility at 20 mg/kg (Fig 3). Hence, this gastrointestinal effect may be mediated, in part at least, by the peripheral CB2 receptor. Administration of the CB2 antagonist SR144528 in part blocked this effect.

Cannabinoids are well known for their cardiovascular activity (for a review see 30). In an outstanding series of papers Kunos and his group have shown that activation of peripheral CB1 receptors contributes to haemorrhagic and endotoxin-induced hypotension, and that anandamide and 2-AG, produced by

macrophages and platelets respectively, may be mediators of this effect.

The hypotension in haemorrhaged rats was prevented by the CB1 antagonist SR 141716A (31,32). Recently the same group found that anandamide-induced mesenteric vasodilation is mediated by an endothelially located SR 141716A-sensitive "anandamide receptor", distinct from the CB1 cannabinoid receptor and that activation of such a receptor by an endocannabinoid, possibly anandamide, contributes to endotoxin-induced mesenteric vasodilation in vivo (33). The highly potent synthetic cannabinoid HU-210, as well as 2-AG, had no mesenteric vasodilator activity (33). Furthermore it was shown that mesenteric vasodilation by anandamide apparently has 2 components: one mediated by a SR 141716-sensitive non-CB1 receptor (located on the endothelium) and the other by an SR 141716A-resistant direct action on vascular smooth muscle (33).

We have reported that the production of 2-AG is enhanced in normal, but not in endothelium-denuded rat aorta on stimulation with carbachol, an acetylcholine receptor agonist (34). 2-AG potently reduces blood pressure in rats and may represent an endothelium-derived hypotensive factor (34). The observations reported now further complicate this already complex picture. We have found that HU-308 reduces blood pressure when administered to rats (Fig 4) and that this cardiovascular effect is blocked by the CB2 antagonist SR 144528, but not by the CB1 antagonist SR 141716A.

Apparently the hypotensive effect caused by HU-308 is produced through a mechanism that differs from the previously described CB1-mediated (or the "anandamide receptor"-mediated) hypotension produced by endocannabinoids. This unexpected observation may serve as a starting point for novel hypotensive drugs, as HU-308 causes no psychotropic effects, as established by the lack of effect in the tetrad of assays described above and therefore we expect that it will not cause major undesirable effects in humans, as most cannabinoids do not produce significant side effects except the psychotropic ones.

HU-308 and indomethacin injected between 30 and 90 min before application of arachidonic acid, induced significant reduction of arachidonic acid-induced ear swelling at a dose of 50 and 20 mg/kg respectively (Fig 5). However the antiinflammatory effect produced by indomethacin was greater than that produced by HU-308. The CB1 antagonist SR 141716A (5 mg/kg) administered 15 min before HU-308, did not prevent the antiinflammatory effect of HU-308. Rather, SR 141716A by itself reduced arachidonic acid-induced ear swelling. The CB2 receptor antagonist SR 144528 (0.5 mg/kg) did not by itself induce an antiinflammatory effect; however it reduced the antiinflammatory effect of HU-308 (Fig 5).

Calignano et al. (20) and Jagger et al. (21) have shown that anandamide attenuates the early phase (20) or the late phase (21) of pain behavior

produced by formalin-induced chemical damage. This effect is produced by interaction with CB1 (or CB1-like) receptors, located on peripheral endings of sensory neurons involved in pain transmission. Palmitylethanolamide, which like anandamide is present in the skin, also exhibits peripheral antinociceptive activity during the late phase of pain behavior (20,21). However palmitylethanolamide does not bind to either CB1 or CB2 (35). Its analgetic activity is blocked by the specific CB2 antagonist SR 144528, though not by the specific CB1 antagonist SR 141716A. Hence a CB2-like receptor was postulated (20).

The results reported now throw further light on the involvement of the CB2 receptor in peripheral antinociception. HU-308 apparently acts through the CB2 receptor as it binds to CB2 but not to CB1. Thus, in our studies, HU-308 reduced peripheral pain during the late phase of pain behavior (Fig. 6) which was prevented by SR 144528, the CB2 antagonist, but not by SR 141716A, the CB1 antagonist. This observation is in agreement with the recent detection by Griffin et al. (9) of CB2 receptors on peripheral nerve terminals. If in the future a CB2-like receptor is indeed identified it will be of interest to investigate the activity of HU-308 on it. Whatever the exact mechanism of the activity of HU-308 on pain transmission, our results indicate that cannabinoids may serve as peripheral analgetics that have no central effects.

In summary, we have synthesized a CB2 specific agonist, code-named HU-308. This new cannabinoid does not bind to CB1 (Ki > 10 mM), but does so efficiently to CB2 (Ki = 22.7 + 3.9 nM). It shows no activity in a tetrad of behavioral tests in mice, which together have been shown to be specific for THC - type activity in the CNS. However, HU-308 reduces blood pressure, blocks defecation and elicits antiinflammatory and peripheral analgetic activity. The hypotension, antiinflammatory, peripheral analgetic activity, and inhibition of gastrointestinal motility produced by HU-308 are blocked by the CB2 antagonist SR 144528, but not by the CB1 antagonist SR 141716A. These exemplary results, which are to be construed in a non-limitative manner, demonstrate the feasibility of discovering novel non-psychotropic cannabinoids that may lead to new therapies for hypertension, inflammation, pain, and gastrointestinal disorders.

The principles of the present invention will be more fully understood in the following examples, which are to be construed in a non-limitative manner.

EXAMPLES

Synthesis and administration of HU-308

The starting materials, 4-hydroxy-myrtenyl pivalate (I) and 5-(1,1-dimethylheptyl)-resorcinol (II) and the intermediate (III) in the synthesis of HU-308 were prepared as previously reported (14). The synthesis of HU-308 is described in Scheme 1. The indicated structure of HU-308,

(structure V) melting point 50°C, [a]D + 127°C (c=2.87 mg/cc CHCl3), was supported by NMR, GC-MS and HRMS data. NMR d 300 MHz, (CDCl3): 6.45 (2H, S, aromatic), 5.7 (1H, olefinic), 4.12 (2H, CH3O-), 4.01 (1H, benzylic), 3.7 (6H, OCH3); HRMS calculated for C27H42O3 414.6287, found 414.3114.

HU-308 was dissolved in ethanol:emulphor:saline(1:1:18) as described previously for other cannabinoids (15,16). HU-308 was administered intraperitoneally (i.p.) into mice in the behavioral, the antiinflammatory and the antinociceptive assays. In experiments where blood pressure was monitored it was administered i.v. into rats. The time schedules and details of administration are indicated in the legends of Figures 2-6.

Receptor Binding Assays.

The CB1 binding assays were performed with synaptosomal membranes prepared from rat brains (3). The CB2 assays were performed with transfected cells (4). The previously described probe [3H]HU-243 was employed in a centrifugation based ligand binding assay (3,17).

Animals and Drugs

Female Sabra mice (2 months old, Harlan-Sprague Dawley, Jerusalem) were used for a series of tests for psychotropic effects (the 'tetrad'), for assessing intestinal immotility ('defecation') and for the assays for inflammation and peripheral pain. Blood pressure was measured in male Sabra rats. HU-308, SR 141716A and SR 144528 (the latter two were a

generous gift of Sanofi Reserche, France) were dissolved in vehicle (ethanol:emulphor:saline=1:1:18) and injected in volumes of 0.1 ml/10g in mice or 0.1 ml/100g in rats.

The pharmacological assays in mice

A series of four consecutive observations are performed on each mouse following a standard procedure employed to evaluate psychoactive cannabinoid-induced effects in mice (15) with similar time intervals as described previously by our group (16). In short, at various times after injection, (see "Results and Discussion"), mice were tested in four assays consecutively: motor activity (ambulation and rearing) in an open field (20 x 30 cm, divided into 12 squares of equal size) for 8 min; immobility ("catalepsy") on a ring of 5.5 cm diameter for 4 min; body temperature with a telethermometer (Yellow Springs Instruments Co.); analgesia on a hot plate maintained at 55 °C was measured as the latency (in seconds) until the first hind paw lick or jump from the plate (the latter response was rarely observed) with a maximum of 45 s.

Inhibition of Intestinal Motility

Immediately after injection of HU-308 (20, 50 or 100 mg/kg), the mice were separated into individual cages and the number of fecal pellets was recorded every 15 min for 2 hours as a measure of intestinal motility. Rectal temperature was also recorded as a measure of central activity.

Arachidonic acid-induced ear inflammation in the mouse

Ear inflammation was measured by assessing ear tissue swelling after topical application of arachidonic acid. Nonsteroidal anti- inflammatory drugs have been shown to reduce swelling in this model (18). Thus at various times after i.p. injections (see Fig 5) of HU-308 (50 mg/kg), arachidonic acid was applied to the inner surface of one ear (4.5 mg in 5 ml ethanol). The opposite ear served as control (5 ml ethanol). Ear thickness was determined (in 0.01 mm units) every 15 min for 90 min starting immediately after arachidonic acid application using a dial thickness gauge (Mitutoyo, Japan).

Peripheral pain

Pain mediated by the peripheral nervous system, was tested in the 'formalin test' for cutaneous (peripheral) pain (19,20,21). First HU-308 (or vehicle) was injected i.p. In experiments which involved an antagonist, the latter was administered i.p. 15 min before HU-308. Formalin was injected s.c. in the hind paw of a mouse 90 min after HU-308. Immediately after formalin administration pain was assessed (every 5 min for 1 hr) by the number of times the animal licks the formalin-injected paw.

Blood pressure assay

Systemic blood pressure was monitored in male rats (Sabra strain, 270-350 g). A chronic cannula (P 50, Clay Adams) was implanted into the femoral

artery under pentobarbital anesthesia (60 mg/kg). The jugular vein was cannulated for drug administration. The arterial cannula was attached to a pressure transducer (Db23, Statham City). The transducer was connected to a data acquisition system (CODAS software and scroller card, Dataq, Ohio) and the pressure was sampled at a rate of 1/s.

Recordings were taken for 30-60 min before treatment. Preliminary observations had indicated that the effects of HU-308 on blood pressure returned to normal well within a 30 min period after administration. Hence, measurements were performed for 30 min following i.v. bolus injections of HU-308. Only one dose of HU-308 (5-40 mg/kg) with, or without antagonist (SR 141716A to block CB1 receptors; SR 144528 to block CB2 receptors) was administered to each rat.

Statistical analyses

Time curves were compared to two-way analyses-of-variance (ANOVA:time versus dose). Differences from vehicle treatments were compared by one-way ANOVA's, followed by post-hoc Newman-Keuls tests (Prism software from Graphpad, San Diego).

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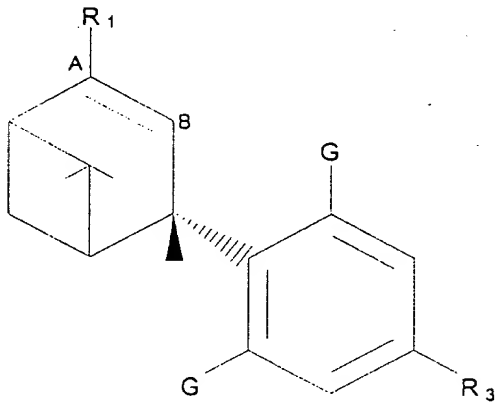
CLAIMS

1. A pharmaceutical composition of matter for treating or preventing hypertension, inflammation, peripheral pain or gastrointestinal disorders, comprising as an active ingredient a compound of the general formula:



having the (3S,4S) configuration, and which is essentially free of the (3R,4R) enantiomer, wherein A---B designates an optional double bond,

R_1 designates a variety of organic moieties,

G designates hydrogen, halogen or various ether groups,

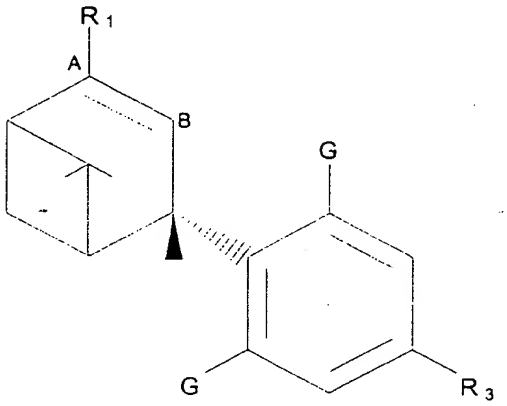
and R_3 designates various alkyl groups, ether groups,

or combinations thereof.

2. The pharmaceutical composition of claim 1 wherein R_1 is CH_2OH, G is hydrogen or OR_2, where R_2 is a lower alkyl group, and R_3 is 1,1-dimethyl heptyl.

3. The pharmaceutical composition of claim 1 wherein R_1 is CH_2OH, G is OCH_3 and R_3 is 1,1-dimethyl heptyl.

4. Use for the preparation of a medicament for treating or preventing hypertension, inflammation, peripheral pain or gastrointestinal disorders, of a compound of the general formula:



having the (3S,4S) configuration, and which is essentially free of the (3R,4R) enantiomer, wherein A---B designates an optional double bond,

R_1 designates a variety of organic moieties,

G designates hydrogen, halogen or various ether groups,

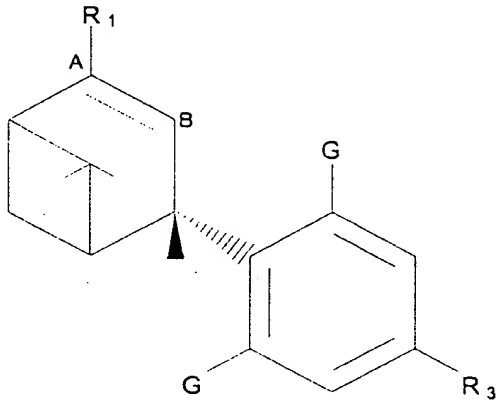
and R_3 designates various alkyl groups, ether groups,

or combinations thereof.

5. Use according to claim 4 of a compound wherein R_1 is CH_2OH, G is hydrogen or OR_2, where R_2 is a lower alkyl group, and R_3 is 1,1-dimethyl heptyl.

6. Use according to claim 5 wherein R_1 is CH_2OH, G is OCH_3 and R_3 is 1,1-dimethyl heptyl.

7. A method for treating or preventing hypertension, inflammation, peripheral pain or gastrointestinal disorders, comprising administering to an individual in need thereof a pharmaceutical composition comprising a therapeutically effective amount a compound of the general formula:



having the (3S,4S) configuration, and which is essentially free of the (3R,4R) enantiomer, wherein A---B designates an optional double bond,

R_1 designates a variety of organic moieties,

G designates hydrogen, halogen or various ether groups,

and R_3 designates various alkyl groups, ether groups,

or combinations thereof.

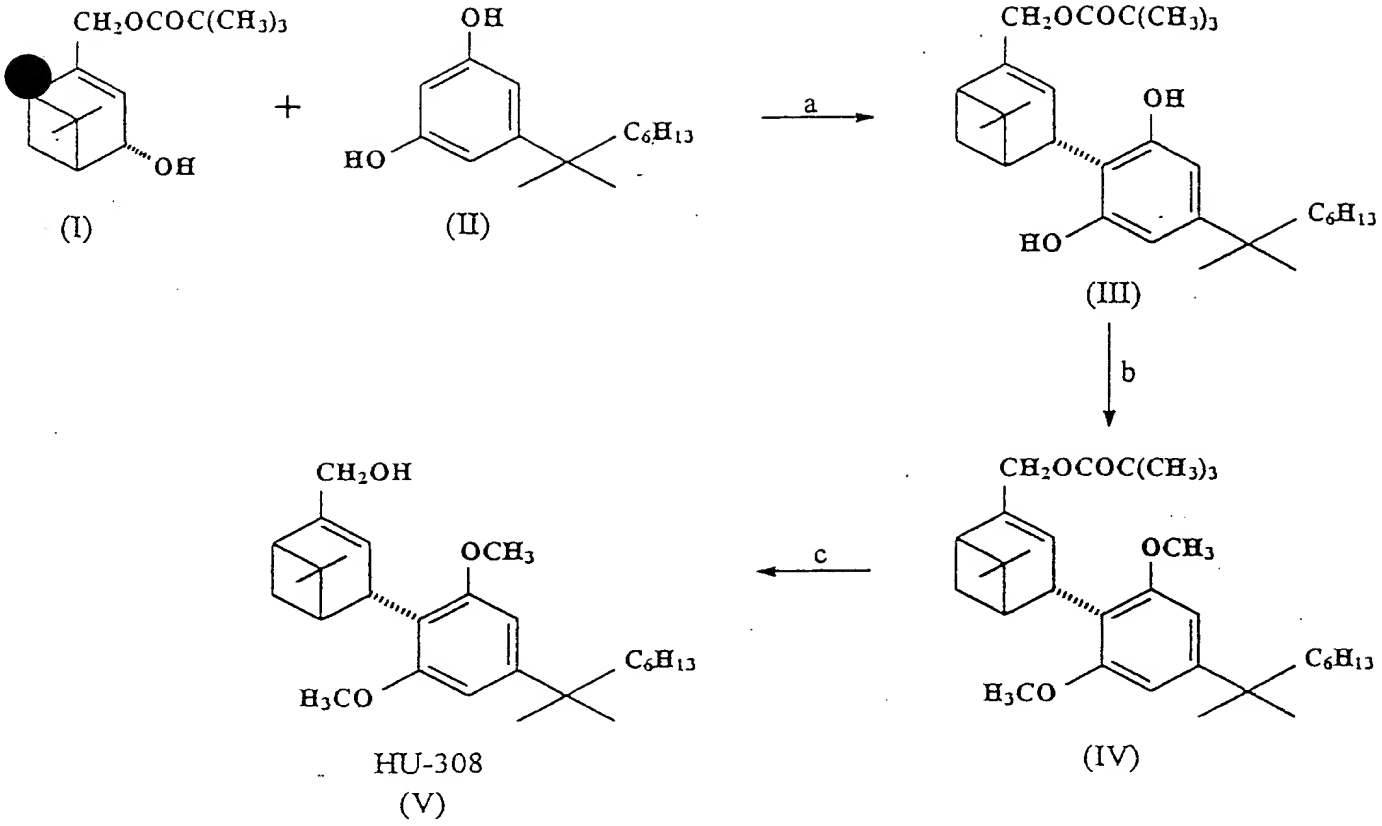
8. The method of claim7 wherein R_1 is CH_2OH, G is hydrogen or OR_2 , where R_2 is a lower alkyl group, and R_3 is 1,1-dimethyl heptyl.

9. The method of claim 8 wherein R_1 is CH_2OH, G is OCH_3 and R_3 is 1,1-dimethyl heptyl.

ABSTRACT

The present invention provides novel pharmaceutical compositions comprising as an active ingredient a compound which is specific for the peripheral cannabinoid receptors. The principles of the invention are disclosed utilizing certain 4-phenyl pinene derivatives. One currently preferred embodiment is a CB2 specific agonist, code-named HU-308. Two types of cannabinoid receptors are known, namely CB1, present in the central nervous system (CNS), and to a lesser extent in other tissues,and CB2 present outside the CNS, in peripheral organs including peripheralnerve terminals. The new cannabinoid HU-308 does not bind to CB1 but does so efficiently to CB2. It shows no activity in a tetrad of behavioral tests in mice which together have been shown to be specific for tetrahydrocannabinol (THC) - type activity in the CNS mediated by CB1. However, HU-308 reduces blood pressure, blocks intestinal motility and elicits antiinflammatory and peripheral analgetic activity. These results demonstrate novel non-psychotropic cannabinoids that may serve as new therapies for hypertension, inflammation, pain, and gastrointestinal diseases.

Synthesis of HU-308



Scheme I.

a: dry p-toluene sulfonic acid in methylene chloride
b: potassium carbonate; methanol
c: lithium aluminium hydride

Figure 1

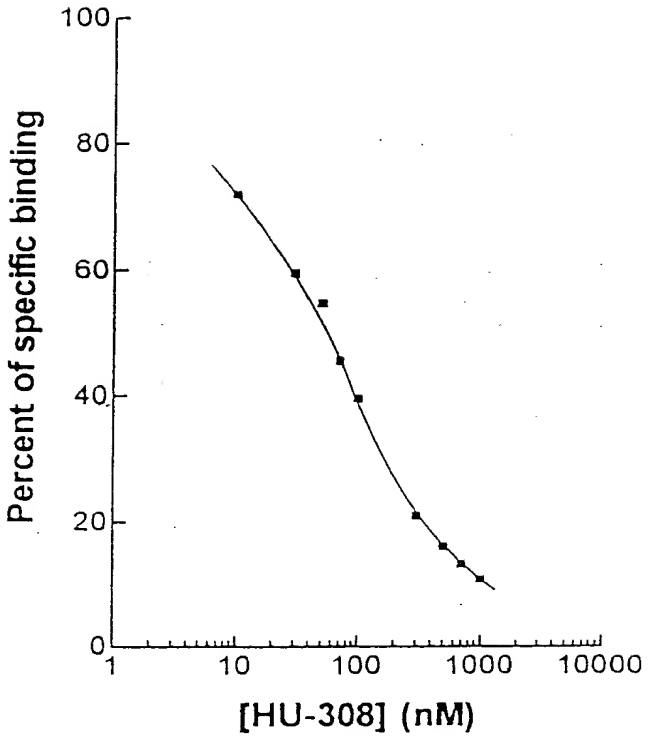
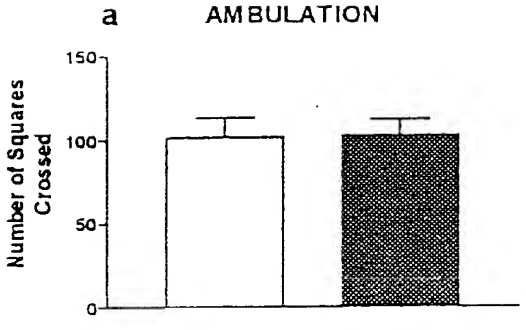
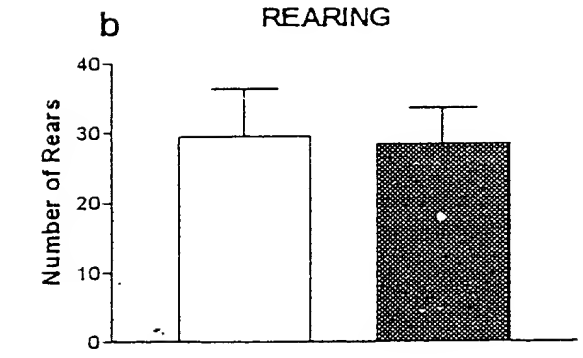
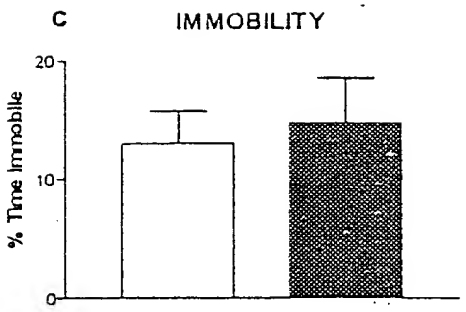
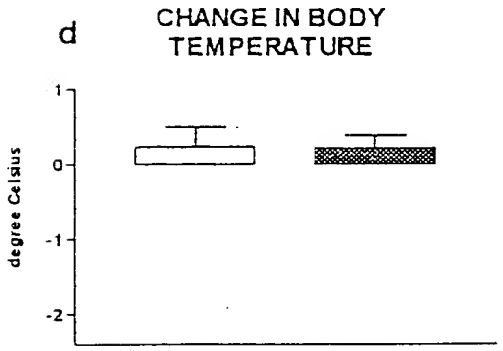

Percent of specific binding
100
80
60
40
20
0
1
10
100
1000
10000
[HU-308] (nM)

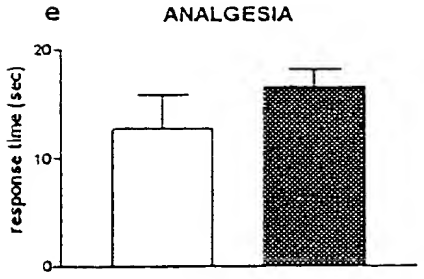
Figure 2


a
AMBULATION
Number of Squares Crossed
150
100
50
0


b
REARING
Number of Rears
40
30
20
10
0


c
IMMOBILITY
% Time Immobile
20
10
0


d
CHANGE IN BODY TEMPERATURE
degree Celsius
1
0
-1
-2


e
ANALGESIA
response time (sec)
20
10
0

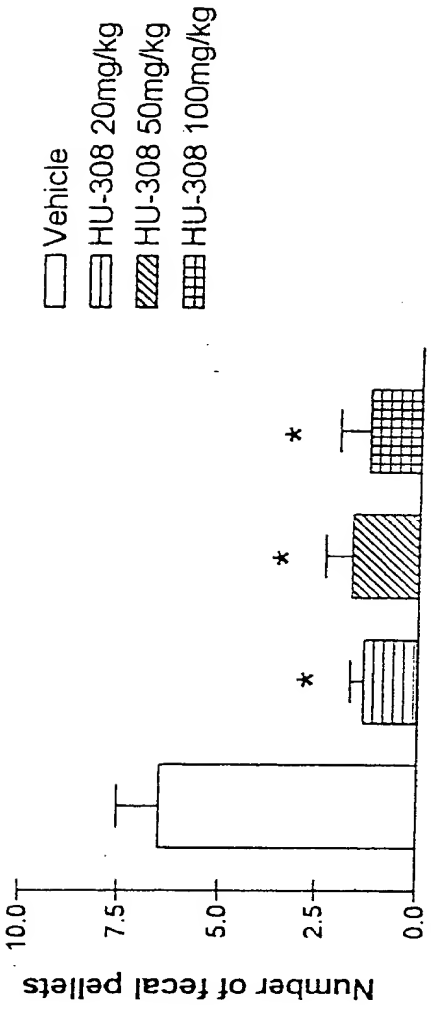

Vehicle
HU-308 20mg/kg
HU-308 50mg/kg
HU-308 100mg/kg
Number of fecal pellets
10.0
7.5
5.0
2.5
0.0
*
*
*

Figure 3

Figure 4

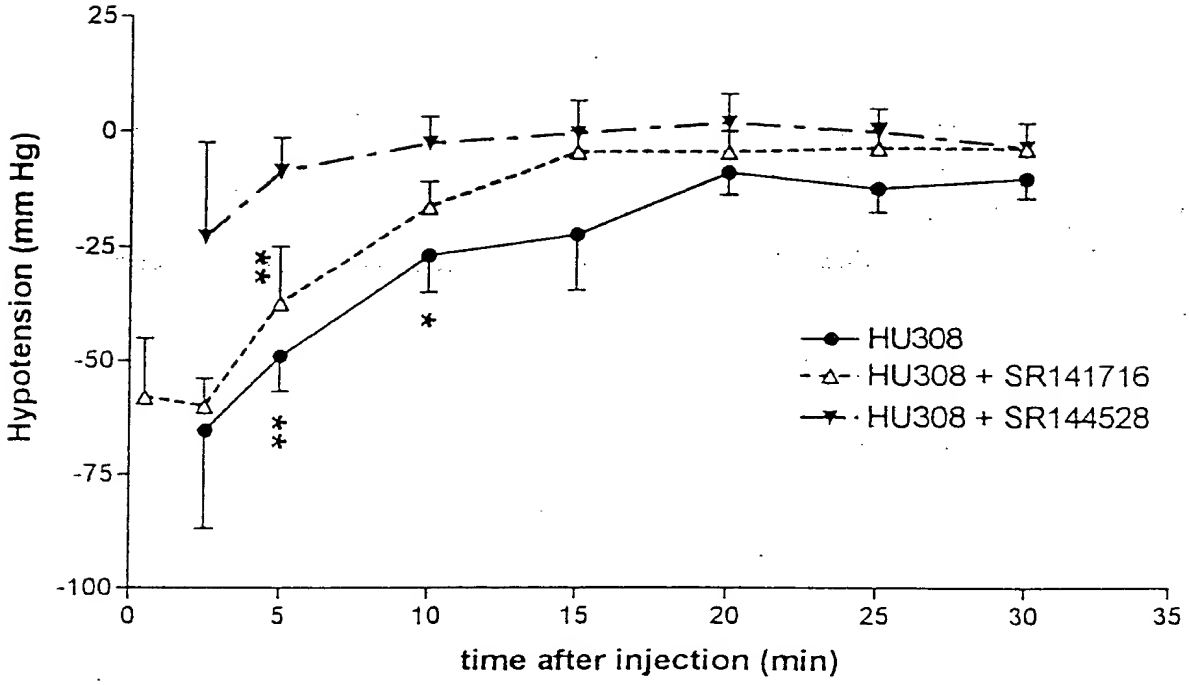
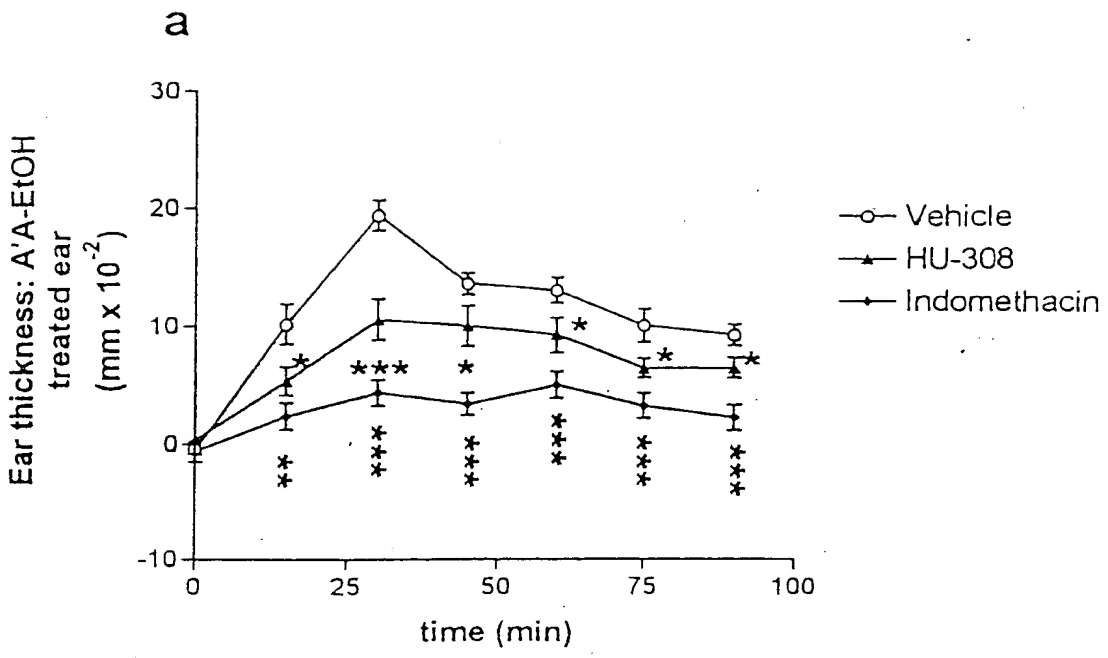
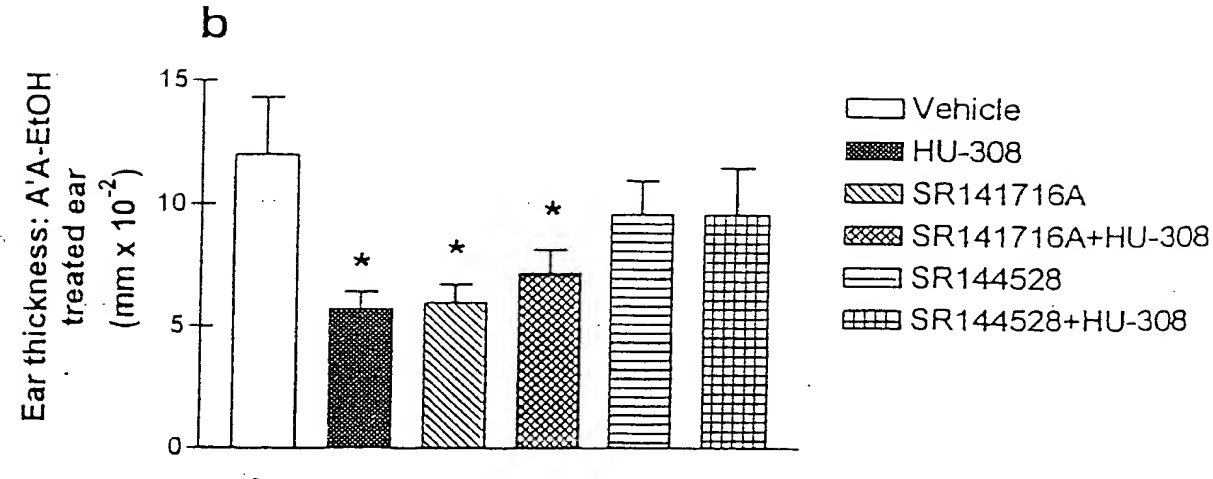

25
0
-25
-50
-75
-100
Hypotension (mm Hg)
0
5
10
15
20
25
30
35
time after injection (min)
HU308
HU308 + SR141716
HU308 + SR144528
**
**
*

Figure 5


a
Ear thickness: A'A-EtOH treated ear (mm x 10-2)
30
20
10
0
-10
0
25
50
75
100
time (min)
Vehicle
HU-308
Indomethacin


b
Ear thickness: A'A-EtOH treated ear (mm x 10-2)
15
10
5
0
Vehicle
HU-308
SR141716A
SR141716A+HU-308
SR144528
SR144528+HU-308